The Impact of Presymptomatic Molecular Testing in Hereditary Cancers

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Molecular Changes Associated with the Development of Cancers

Introduction

Genetic factors are likely to be the primary determinants in around 5–10% of cancer patients. Over the last decade the identification of some of these cancer susceptibility genes has revolutionised our understanding of cancer in general. In addition, it has allowed us to improve our management of patients in those families with a genetic predisposition by offering presymptomatic testing and hence early intervention and treatment for the disease. This review will examine the major hereditary cancer syndromes and look at the tests currently available for their diagnosis.

The development of any tumour is a multistage process involving mutations in a number of genes. Some mutations are found in genes which promote cell proliferation, increasing the target cell population and thus making it more likely that subsequent changes will occur. Other mutations affect the stability of the genome e.g. by affecting genes associated with DNA repair processes and thereby increase the overall mutation rates. In the 1950s it was estimated that 6–7 successive changes would be required to convert a normal cell into a malignant one. This explained in part why the majority of cancers are diseases of older age groups, as it will take some time for each of these steps to occur. In inherited cancers, the first mutation is inherited in the germ line hence the cancers are likely to occur at an earlier age. Several groups of genes are associated with cancers, most notably the oncogenes and tumour suppressor genes [1].

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Oncogenes

These genes belong to groups of genes which in their normal state, termed proto-oncogenes, control cell proliferation. The protein products of the proto-oncogenes function at all levels of the cell and belong to one of five groups: growth factors, factor receptors, components of the signal transduction pathways, DNA binding proteins or proteins controlling the cell cycle. Mutation of the genes occurs either through point mutation, amplification, chromosome translocation leading to production of fusion genes or chromosome translocations resulting in loss of control of gene expression. Each of these mutations are gain of function mutations, resulting in inappropriate or excessive expression of the gene [1]. These changes act in a dominant manner with only one mutation in the gene being necessary. Many different oncogenes have been identified in a wide range of tumours. Initially it was believed that oncogenes would not be associated with inherited cancers as they were thought to be lethal. There is however one exception to this, the RET oncogene, which causes multiple endocrine neoplasia, which is discussed below.

Tumour Suppressor Genes

The existence of this group of genes had been known for many years. Experiments indicated that the normal phenotype could be restored by fusion of a malignant cell with a normal one [1]. This provided the first evidence for recessive, loss of function mutations in genes. Direct evidence for a tumour suppressor gene came from studies of the rare childhood tumour retinoblastoma. In 1971, Knudson [1] proposed that two 'hits' in the retinoblastoma gene would be required before the tumour could develop. In the sporadic form of the condition, two separate mutations would be necessary for tumour development and hence it was likely that the disease would

occur at a later age and would be unilateral. In the familial form of retinoblastoma, one mutation would be present in all cells of the body as it is inherited in the germ line and hence earlier ages of onset and the frequent occurrence of bilateral tumours would be more likely. This is in fact what is seen in sporadic and familial retinoblastomas. Molecular studies during the 1980s confirmed this two-hit hypothesis using the so-called loss of heterozygosity test. DNA from both blood and tumour were compared, using polymorphic markers from a region close to the retinoblastoma gene on chromosome 13. Markers which were heterozygous in DNA from normal tissue were shown to be apparently homozygous in tumour DNA. In fact, the DNA from the tumour contained only one mutated copy of the gene and the second copy of the gene had been lost. Hence the polymorphic marker was present in the hemizygous state. This confirmed Knudson's two-hit hypothesis. The first hit in the gene was usually a point mutation and the second hit, occurring in the tumour, was usually a deletion of the second copy. This therefore also led to loss of one copy of the polymorphic marker resulting in loss of heterozygosity. This type of analysis with markers from throughout the genome led to the identification of further tumour suppressor genes, many of which were shown to be the cause of hereditary cancers. In each case, the earlier onset of inherited disease could be explained by the existence of one mutation in all cells of the body with a second hit in the wild-type copy of the gene occurring in the majority of cases. Although these mutations are recessive at the level of the cell, the cancers are dominantly inherited as the second hit is almost certain to occur and it is rare for a tumour not to develop.

Table 1. Hereditary cancers and their associated genes

Disease	Gene	Major associated cancers
Familial adenomatous polyposis	APC	colorectal
Hereditary non-polyposis colon cancer	MSH2/MLH1 PMS1/PMS2 MSH6	colorectal, endometrial, small intestine, hepatobiliary, pancreatic, ureter and renal pelvis
Breast/ovarian cancer	BRCA1, BRCA2	breast, ovarian, pancreatic, laryngeal
Von Hippel-Lindau	VHL	retinal angioma, renal cell cancer, haemangioblastoma, pheochromocytoma
Gastric cancer	CDH1	gastric cancer
Multiple endocrine neoplasia 1	MEN1	parathyroid, anterior pituitary, endocrine pancreas
Multiple endocrine neoplasia 2	RET	medullary thyroid cancer, pheochromocytoma
Gorlin's syndrome	PTCH	basal cell carcinomas
Peutz-Jeghers syndrome	LKB1	intestinal hamartomas, colorectal cancer, breast cancer
Cowden's disease	PTEN	skin and mucous membrane hamartomas, breast cancer
Renal cell cancer	MET	renal cell cancer

Inherited Cancer Syndromes

Inherited cancer syndromes can be recognised by a number of features such as an earlier age at onset than is normal for the cancer, the presence of multiple tumours or bilateral disease, a familial clustering of cancers and segregation of the disease in a Mendelian manner. Some of the hereditary conditions and their associated genes are listed in table 1.

Recognition of a genetic predisposition in a family means that they can be offered clinical screening. However, in the majority of these syndromes, the disease segregates in an autosomal dominant manner, which means that 50% of the offspring will develop the disease and 50% will not. Screening by clinical methods necessarily means that all individuals will have to have regular invasive procedures which can be unpleasant and stressful for the patient as well as costly for the provision of health services. The identification of

the genes causing many of the inherited cancers means that we can now adopt a more targeted approach to helping each family. Once a family has been identified as having an inherited predisposition to cancer, the causative gene or genes can be screened and the mutation responsible identified. At-risk family members can then be offered a test to determine if they have inherited the defective gene and if they have, they can then continue to be screened clinically and treated at an early stage before the cancer develops. Those who have not inherited the defective gene need no longer be included in the screening programme.

Procedures for Genetic Testing

Genetic testing for inherited cancers requires appropriate indications and a testing strategy plus provision of both pre- and post-test counselling. For this reason, it is generally

recommended that testing should be carried out within the structure of a genetics centre where multidisciplinary teams are available to provide both clinical and scientific expertise [2, 3]. Issues which need to be considered are:

(1) A full family history should be available so that a pedigree can be drawn up. It is often necessary to confirm diagnoses on deceased family members by obtaining pathology reports or obtaining details from cancer registries. It is not unusual to get information from family members regarding deceased relatives which when followed up proves to be incorrect. The accuracy of family information has been studied and shown to be quite variable particularly depending on the type of cancer and the degree of kinship of the affected relative [4].

(2) Families need to be given both the clinical and scientific background to the cancer and should be made aware of all their options. Some families may not want molecular testing and may prefer to remain in a clinical screening programme. In some families, it may not be possible to obtain a mutation result so they should be counselled with this in mind. Counsellors should not be directive when discussing options with patients but should instead be able to provide individuals with all the information which will enable them to make an informed decision on issues.

(3) Families need to be clear about their risks. Attitudes to risk will differ from one family to another, as what one person perceives as high risk may be an acceptable risk to someone else.

(4) Appropriate support should be available both to deliver results of molecular tests and to follow up individuals after testing. Support is often still necessary if an individual gets a negative test. Although the majority of individuals are likely to be happy with a negative result, which puts their own off-

spring in the clear, the phenomenon of survivor guilt is also well recognized [3].

Other issues are also important. There are a number of ethical issues which should be considered before predictive testing is carried out. One of these is the age at which testing should be done. In general, testing should not be carried out on children until they are at an age to understand the process and make their own decision as to whether they want the test [5]. Incorporation of a positive result in a child's medical record could for example also lead to problems in later life such as with employment and insurance issues. However, some conditions, such as familial adenomatous polyposis, develop during adolescence, and it may therefore be appropriate to carry out the testing in childhood [2, 6, 7]. In other conditions such as hereditary non-polyposis colon cancer, the majority of individuals do not develop the disease until they are in their 20s and 30s, so predictive testing can be delayed until individuals reach adult life. Screening options in some inherited cancers may be straightforward, as in hereditary colon cancers, in which a premalignant lesion can be identified at an early stage and removed or appropriate surgery can be carried out. A more difficult issue to address is how to deal with situations for which clinical screening is not proven, e.g. in Li-Fraumeni syndrome. This condition is associated with a wide spectrum of tumours (see below) for which effective screening is not readily available. In this situation, it may be that individuals may prefer to be aware of their risks so that they can plan their life and make informed decisions about issues such as childbearing even though the available clinical screening may not be able to help them directly. Similarly in familial breast cancer, mammography in premenopausal women is not entirely efficient. In addition it is now clear that the penetrance of some genes such as the BRCA1 and 2 genes

associated with familial breast and ovarian cancer is not 100%. Women therefore have to be made fully aware of their options before deciding on a treatment such as prophylactic mastectomy [8]. Finally, the option of prenatal diagnosis needs careful consideration for a condition with adult onset and for which treatment such as prophylactic surgery can potentially offer at least a partial cure.

As well as all the clinical considerations, it must be remembered that the knowledge that an individual carries a mutation in a gene, which predisposes him or her to develop cancer, has major implications for the ability to obtain life or health insurance. A survey of members of genetic support groups with a variety of genetic disorders in the family indicated that 25% of them were refused life insurance, 22% were refused health insurance and 13% were denied or lost employment [9]. A survey of insurance companies showed that 10% would not provide health insurance to HNPCC (hereditary non-polyposis colon cancer) gene carriers and 20% said that they would increase the premiums. A quarter of insurers would not provide life insurance or would only provide it at increased premiums [10]. Therefore, although there are considerable benefits to offering molecular testing for inherited cancers, there are many issues which should be considered before undertaking such a process.

This review will consider what is currently available for the molecular testing of familial cancer syndromes beginning with a brief description of the molecular methods used for mutation detection.

Methods of Mutation Detection

The basic strategy for testing for mutations in each of the familial cancer syndromes is similar although the specific methodology

may vary. It is complicated by the fact that for each of the genes associated with the familial cancers described in subsequent sections, many different mutations have been described, the majority having been found in only one family with the condition. This makes the situation far more complicated than for genetic diseases, such as sickle cell disease, where all affected individuals carry the same mutation. In addition cancer genes tend to be large and the associated mutations involve only a single or a few bases and hence can be difficult to find.

In each family, DNA from an affected individual should always be tested in the first instance to identify the causative mutation [7]. If there is no living affected individual in a family from whom DNA can be obtained, it may still be feasible to carry out mutation detection if formalin-fixed paraffin blocks of tissue removed at the time of surgery are available from which DNA can be extracted [11]. Unaffected at-risk individuals should not be tested until the mutation is identified since no method of mutation detection is 100% sensitive. The absence of an identifiable change in an at-risk individual does not therefore necessarily mean that the individual is no longer at risk.

All mutation detection methods are time-consuming and expensive and there is no single perfect method. Even the gold standard, direct sequencing of the gene, can miss mutations. The specific method chosen to screen for mutations is dependent on a number of variables including (a) sensitivity, (b) budget, (c) hazards associated with chemicals required, (d) technical ease, (e) nature of mutations associated with the disorder and (f) personal experience. Only a brief description of the commonest methods is given here. Full details of them can be found in Langren [12] and Cotton et al. [13]. In general, each of the scanning methods described below entails

Fig. 1. An example of SSCP of exon of 12 hMSH2. Abnormal bands are seen in lanes 4, 5, 8, 9. Lanes 2 and 6 are blank. All other lanes show a normal pattern. (Courtesy of Yvonne Wallis, West Midlands Regional Genetics Laboratory.)

analysis of a gene, exon by exon, to identify the presence of possible sequence variation. In each case, an exon or fragment of an exon if it is large, is amplified by the polymerase chain reaction (PCR) and the products are then analysed by gel electrophoresis. The exon or exons identified as potentially abnormal are then sequenced.

Single-Stranded Conformation Analysis (SSCP)

This is a relatively straightforward technique, which is widely used. DNA is firstly amplified by PCR, then denatured to produce single-stranded molecules and electrophoresed through an acrylamide gel, which is subsequently stained – usually by silver staining – to visualise the DNA fragments. Alternatively, fluorescently tagged primers can be used at the PCR stage and the fragments are then detected by electrophoresis on an automated

sequencer. Even single nucleotide changes are sufficient to influence the secondary structure of a single-stranded piece of DNA, which will in turn alter its migration pattern through the gel. The presence of a sequence variation is therefore detected by the mobility shift of the fragment (fig. 1). As both neutral polymorphisms as well as true mutations will cause such shifts, any fragment of the gene which does show a change must be sequenced to confirm that the alteration is a mutation. The main advantage of SSCP is its simplicity with relatively low costs. The main drawback of the technique is its limited sensitivity. If the DNA fragment under investigation is relatively short, e.g. less than around 300 bp, the sensitivity is good at over 80%. However, the larger the fragment, the lower the sensitivity and in fragments over 500 bp the detection rate can drop below 40%.

Heteroduplex Analysis

This technique is similar to SSCP but here the DNA fragments are run in double-stranded form. Following PCR, the DNA is denatured, then allowed to reanneal slowly. Two DNA strands derived from two different alleles can reanneal and will result in a molecule which is mismatched at the site of sequence variation. This heteroduplex molecule will migrate differently to the wild type or mutant homoduplex molecules which are perfectly matched, and this can be detected as a mobility shift on the gel. This technique is therefore also very easy to carry out, but as with SSCP it lacks sensitivity. In general, it can detect insertion and deletion mutations with greater sensitivity than point mutations as the mismatched regions are larger.

Denaturing Gradient Gel Electrophoresis (DGGE)

This technique makes use of the fact that as DNA is electrophoresed through a gradient

of increasing denaturant, it will eventually become single-stranded. The concentration of denaturant at which this occurs is dependent on the sequence of the DNA. It therefore follows that two DNA species with differing sequence will denature at different concentrations. As a region of the DNA fragment becomes single-stranded and the molecule becomes branched, its migration through the gel is retarded. As with the techniques above, the presence of sequence variation can be identified by a mobility shift in the PCR fragment after the gel has been stained (fig. 2). The major advantage of DGGE is that it is close to 100% sensitivity. It can, however, be quite time-consuming to establish, as each PCR fragment will melt at a differing range of denaturant and this has to be determined before the technique can be used. It can also be expensive as the PCR primers have a 'so-called' GC clamp – approximately 40 bp of GC sequence – attached to each primer. This region has a very high melting temperature, which will ensure that the PCR fragment will not become completely denatured during its migration through the gel. Should the fragment become completely single-stranded each molecule would migrate at a similar rate and it would be impossible to differentiate mutant from wild-type sequence.

Protein Truncation Test (PTT)

This test makes use of the fact that many causative mutations in the cancer genes are nonsense mutations, i.e. mutations which result in the replacement of a codon for an amino acid with one for a stop codon. This in turn results in a prematurely truncated protein. The basis of the technique is to carry out an in vitro transcription and translation reaction. DNA is amplified using primers which contain a T7 promoter sequence and a eukaryotic translation initiation sequence. After the PCR reaction, the products can therefore be tran-

Fig. 2. DGGE analysis of exon 8 of APC. The wild-type homoduplex band is seen in all tracks. Mutant homoduplex bands are seen immediately above (lane 6) or below (lanes 9–12). Heteroduplex bands are seen above the homoduplex bands in lanes 6 and 9–12. Lanes 1 and 8 are blank. (Courtesy of Yvonne Wallis, West Midlands Regional Genetics Laboratory.)

scribed into RNA and subsequently translated into a peptide sequence reflecting part of the protein under investigation. In order to detect the in vitro synthesized peptide, radio-labelled amino acids are included in the translation reaction. The translated products are then resolved on an SDS polyacrylamide gel. The appearance of a shorter than normal peptide indicates the presence of a nonsense mutation (fig. 3). The great advantage of this technique, unlike those above, is that it will only detect true mutations and not polymorphisms. In addition, the initial PCR reaction is carried out over a region of up to 1 kb instead of a few hundred base pairs. This means, for example, that exon 15 of the APC gene (see below) can be analysed in 4 segments instead of the 23 reactions normally used if analysed by SSCP or DGGE [14]. The disadvantage of PTT is that it cannot detect missense mutations, i.e. mutations which result in the alternative of one amino acid for another, which are causative in some genes, e.g. the VHL gene. In addition, RNA may be needed for the starting material if the target gene contains many small exons interrupted by large stretches of intronic sequence which are hence too large to amplify by PCR. In this

Fig. 3. PTT analysis of exon 15 of APC. Wild-type bands are seen at the top of each gel. Truncated proteins are detected as additional smaller bands in lanes 3, 11, 12 and 13. (Courtesy of Yvonne Wallis, West Midlands Regional Genetics Laboratory.)

situation a cDNA template has to be made from RNA as starting material for the PCR reaction. Only in genes with large uninterrupted regions of coding sequence, e.g. APC, exon 15 or exons 10 and 11 of BRCA1 and BRCA2, can genomic DNA be used as a template.

Direct Sequencing

Direct sequencing of the genes is perhaps the most sensitive of all methods for detection of mutations. In general, both forward and reverse strands of the gene are sequenced. It is

now most commonly carried out according to the dideoxy method of Sanger by PCR using either fluorescently labelled dideoxy chain terminators or primers. The products can then be detected on commercially available DNA sequencers. The obvious advantage of the technique is that it should theoretically pick up all mutations. However, it is possible to miss point mutations present in the heterozygous state. The major disadvantages of the technique are the time-consuming nature of the test, the high cost of the procedure and the availability of considerable time on a sequencer.

Other Techniques

In addition to the methods described above, RNase protection, bi-dideoxy fingerprinting and chemical cleavage of mismatches have also been used for the detection of mutations in cancer genes. Recently, denaturing high performance liquid chromatography has been introduced into laboratories and shown to be a potentially useful and sensitive alternative to the methods described above.

DNA 'chips' or microarrays are increasingly being developed as an alternative approach to the detection of mutations. These 'chips' consist of microarrays of hundreds to thousands of oligonucleotides fixed onto a glass slide. For detection of mutations in a cancer gene, the oligonucleotides spotted onto the slide correspond to all wild-type and single nucleotide substitution sequences in the gene of interest. The technology then makes use of a hybridisation approach. Target DNA from the patient under investigation is amplified by PCR, fluorescently labelled and hybridised to the oligonucleotides attached to the slide. If the patient's DNA contains a mutation, it will hybridise to the complementary oligonucleotide on the slide and can be detected by fluorescence-based detection methods. This has been trialed with the BRCA1 gene associated

with familial breast cancer. However, there are still problems with the technology, particularly with respect to its specificity, so the true potential of the technique remains to be realised.

Familial Colon Cancer

In the USA around 150,000 cases of colorectal cancer are diagnosed each year and it is the cause of death in around 60,000. In the UK, it accounts for around 15,000 deaths per annum and as such, the disease remains a major cause of morbidity and mortality in the Western world. Ten to fifteen percent of cases of colorectal cancer will have a hereditary component. The underlying mechanism in many of these remains unknown, but in recent years it has been well established for two conditions. These are familial adenomatous polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC), which together account for up to 5% of cases of colorectal cancer. Mutations in the causative genes for these two diseases place mutation carriers at a lifetime risk of developing colorectal cancer of 80–100% [15]. Both conditions are inherited in an autosomal dominant manner and thus 50% of the offspring of an affected parent are at risk of developing the disorder. The identification of the genes responsible for these symptoms means that it is now possible to offer patients presymptomatic diagnosis and assist in the early removal of potentially malignant lesions preventing the development of cancer and the early death of the individual.

Familial Adenomatous Polyposis

FAP accounts for around 1–2% of all colorectal cancer. It is characterised by the presence of hundreds to thousands of polyps throughout the colon and rectum and the

presence of these features can be used to confirm the diagnosis. Without surgical intervention it is close to 100% certain that at least one of these polyps will become malignant, usually by the third or fourth decade of life. Until recently, cancer was often already present by the time the patient was investigated and frequently led to the early death of the patient [16]. In addition to the colorectal symptoms a number of extracolonic features are associated with the disease including osteomas, epidermoid cysts, congenital hypertrophy of the retinal pigment epithelium and desmoid tumours. Upper gastrointestinal adenomas are also found as well as hepatoblastomas, periampullary cancers and thyroid cancers [17]. Approximately 20% of cases of FAP are due to new mutations.

Prevention from early death from colorectal cancer requires reliable screening procedures. The recommended treatment for FAP is generally a total colectomy with ileorectal anastomosis. Subsequent monitoring should then include lifelong surveillance of the rectal stump and regular upper gastrointestinal surveillance. At-risk individuals should be monitored by regular, usually annual, sigmoidoscopy from the early teens until at least 40 years of age [18].

The gene for FAP, the APC gene, was localised to the long arm of chromosome 5 in 1987 [19] and was isolated 4 years later [20, 21]. A coding sequence of 8,532 bp was identified divided into 15 coding exons (fig. 4). The first 14 exons are several hundred base pairs whereas exon 15 is unusually long, encompassing 6.5 kb. An additional coding exon known as exon X or 10A was subsequently isolated [22]. The protein encoded by APC has a number of functions including a role in cell adhesion through its association with β -catenin and subsequent interaction with the cell adhesion molecule E cadherin [23]. APC protein also functions in various signal trans-

Fig. 4. Structure of the APC gene and APC protein. Genotype-phenotype correlations are indicated. (Reproduced with permission of Dr. R. Fodde and Begell House Publishers.)

duction pathways including the Wnt signaling pathway [24].

The isolation of the gene enabled direct mutation analysis of the families to be carried out. Many mutations have so far been identified [25, 26] and are spread throughout the coding region of APC. The vast majority, >99%, of mutations are truncating mutations caused either by point mutations or small insertions or deletions. No missense mutation has as yet clearly been shown to be the cause of the disease although some have been implicated as predisposing to colon cancer [27, 28]. The majority of mutations have only been seen in a single family. However, two mutations have been found world-wide and account for up to 10% of cases of FAP. These are both 5-bp deletions at codon 1061 and codon 1309. (A database of APC mutations

can be found at <http://perso.curie.fr/Thierry.Soussi/APC.html>.)

Mutation analysis of DNA from an affected individual normally depends on the use of a technique such as SSCP or DGGE to study the first 14 small exons and PTT is most commonly used to study exon 15 in four overlapping fragments. The two common mutations can be screened for initially by direct PCR [25]. Even if affected individuals are deceased, it is still possible to obtain mutation results, as DNA extracted from tumour blocks has been shown to be suitable for PCR analysis [11]. A germ line mutation has been identified in 20–90% of FAP patients in published studies [25, 26, 29]. The variation in pick-up rates reflects the extent to which the gene has been analysed and the technique used. It is also likely to reflect the accuracy of the clini-

cal diagnosis of the patient referred, as, although the majority of cases can be confirmed by the histological appearance of the colon, there is some variation in phenotype both in number of polyps, age at onset of the disease and presence of extracolonic features.

Several studies have examined genotype-phenotype correlations in APC (fig. 4) [25, 30, 31]. An early association was made between the position of mutation and the presence or absence of congenital hypertrophy of the retinal pigment epithelium [30, 32]. Desmoid tumours are primarily, although not solely, associated with mutations at the 3' end of the gene [33, 34]. Mutations from codons 1250 to 1464 have been associated with a profuse phenotype with more than 5,000 polyps present [35] and the commonly seen mutation at codon 1309 is associated with a severe phenotype [36]. Attenuated FAP is associated with mutations near the 5' end of the gene [37]. However, these findings are not absolute, and so cannot be used to predict the phenotype in patients with any certainty. For example, a particularly severe phenotype has been seen in two families with mutations in exon 11. In these families, affected individuals were diagnosed as young as 5 years of age and cancer was detected in 1 individual aged 9 [7]. The presence of extracolonic manifestations can be used, however, to indicate where to look initially for mutations [30]. The complex relationship between genotype and phenotype is made even more complex by the identification of possible modifiers of the phenotype. Modifier loci in FAP were first identified by investigation of the MIN mouse – a mouse which develops multiple intestinal polyps and which has truncating mutations in APC [38]. A candidate gene for the modifier has been proposed to be the secreted form of phospholipase A2, which is found on chromosome 1p in humans [39].

Hereditary Non-Polyposis Colon Cancer

HNPCC is more common than FAP, accounting for 3–5% of colorectal cancer. It is subdivided into three clinical forms called Lynch type 1, Lynch type 2 and Muir-Torre syndromes. As with FAP, it is associated with colorectal cancer, but in this condition the mean age at onset is later than in FAP at around 45 years of age. The lifetime risk of developing colorectal cancer in gene carriers is around 80%. Other features include (1) predominantly right-sided tumours, (2) the presence of synchronous and metachronous tumours, (3) tumours with poor differentiation and (4) tumours which behave rather more indolently than other colorectal cancers [40]. In Lynch type 2 syndrome as well as colorectal cancer, there is an increased risk of other cancers such as endometrial, ovarian, stomach, small bowel and hepatobiliary cancers as well as transitional cell tumours of the renal pelvis and ureter. In Muir-Torre syndrome all of these cancers are found along with sebaceous adenomas, carcinomas and keratoacanthomas (fig. 5). Treatment for HNPCC-affected individuals is generally subtotal colectomy with ileorectal anastomosis or hemicolectomy with surveillance of the remaining bowel. Because of the high risk of endometrial cancer, prophylactic hysterectomy has been proposed once an individual's family is complete. The alternative is regular pelvic ultrasound. At-risk individuals require colonoscopy from 20–25 years of age at 2- to 3-yearly intervals and ultrasound screening of women at risk should also be offered [41].

Unlike FAP, HNPCC cannot be distinguished purely on clinical features. It was originally defined by a series of criteria termed the Amsterdam criteria, which were described to facilitate research before the isolation of the causative gene [42]. These criteria are (1) histologically verified colorectal cancer in at least 3 family members, 1 of

Fig. 5. Pedigree of a family with the Muir-Torre variant of HNPCC indicating the variety of tumours seen in affected individuals. Males are shown as squares and females as circles. Affected individuals are indicated by filled-in symbols. Deceased individuals are indicated by a line through the symbol.

whom is a first-degree relative of another, (2) 1 individual must have been diagnosed under 50 years of age, (3) colorectal cancer should involve at least two generations and (4) FAP must have been excluded. However, the Amsterdam criteria do not take into account other features of the disease, such as extracolonic cancers, and are now generally considered too strict for assessing families suitable for molecular testing now that the genes have been identified. These less strict criteria are termed the Bethesda criteria [43] and include characteristics reported in HNPCC as shown in table 2.

A gene for HNPCC was mapped to chromosome 2p in two large kindreds following a

genome-wide search with 345 microsatellite markers [44]. At that time it was clear that there was genetic heterogeneity and indeed within a few months a further family was shown to be linked to a gene on chromosome 3p [45]. The majority of families with HNPCC were believed to be linked to either chromosome 2p or 3p, but at least one family was shown to map to neither locus.

At the same time as linkage studies to map the genes were ongoing, further studies were identifying a surprising phenomenon. When the DNA from tumours was analysed by PCR amplification of microsatellite markers alongside DNA from normal tissue from the same patient, shifts in the electrophoretic mobility

Fig. 6. Example of microsatellite instability using the BAT 40 marker. For each patient the left-hand lane shows the result obtained using DNA from normal tissue (N) whereas the right-hand lane shows the result from tumour DNA (T). Patient 1638 shows microsatellite instability in the tumour. (Courtesy of Prof. E. Maher, Division of Molecular and Medical Genetics, Department of Paediatrics, University of Birmingham.)

Table 2. Bethesda criteria for the diagnosis of HNPCC

- 1 Individuals with cancers in families that meet the Amsterdam criteria
- 2 Individuals with two HNPCC-related cancers, including synchronous and metachronous cancers or associated extracolonic cancers
- 3 Individuals with colorectal cancer and a first-degree relative with colorectal and/or HNPCC-related cancers and/or a colorectal adenoma; one of the cancers diagnosed <45 years and the adenoma diagnosed <40 years
- 4 Individuals with colorectal cancer or endometrial cancer diagnosed at age <45 years
- 5 Individuals with right-sided colorectal cancer with an undifferentiated pattern on histopathology diagnosed at age <45 years
- 6 Individuals with signet ring cell type colorectal cancer diagnosed <45 years
- 7 Individuals with adenomas diagnosed at age <40 years

of the markers were seen (fig. 6). These shifts can be seen as the presence of additional bands in the tumour DNA. This was taken to mean that there was genomic instability manifesting as errors in the replication of DNA in the tumours [46]. The phenomenon was termed replication errors or microsatellite instability (MSI). MSI has been identified in nearly all the tumours of patients with HNPCC both colorectal and extracolonic, but has also been seen in 10–15% of sporadic colorectal cancers [47]. MSI had previously been identified in micro-organisms, both *Escherichia coli* and *Saccharomyces cerevisiae*, and this was known to be due to defects in the proteins of the mismatch repair pathway [48] (table 3). These proteins function by increasing the fidelity of replication by repair of DNA polymerase misincorporation errors. It was therefore hypothesized that there might be human homologues of the bacterial/yeast mismatch repair proteins, which when mutated gave rise to the replication errors seen in the tumours and might therefore be the genes associated with HNPCC. The human homologue of the *E. coli* gene, *mutS*, was mapped to chromosome 2p [49] and identification of mutations in this gene, called hMSH2, in kindreds with HNPCC confirmed that it was one of the causative genes. Mutations in the human homologue of the *mutL* gene, hMLH1, were identified shortly after [50]. Three more human mismatch repair genes, hPMS1, hPMS2 and hMSH6 or GTBP, all of which are mutated in some HNPCC families, were subsequently identified [51, 52]. Table 3 gives details of these mismatch repair (MMR) genes.

hMSH2 and hMLH1 account for over 90% of cases of HNPCC and so together are the two genes which are screened routinely in diagnostic laboratories using the mutation detection methods described above [53, 54]. Details of mutations identified can be found

Table 3. Mismatch repair genes associated with HNPCC and their relationship to yeast and bacteria

Gene	<i>E. coli</i>	<i>S. cerevisiae</i>	cDNA, bp	Location
hMSH2	mutS	MSH2	3,111	2p16
hMLH1	mutL	MLH1	4,244	2p16
hPMS1	mutL	PMS1	2,484	3p21-23
hPMS2	mutL	PMS1	3,063	2q31-33
hMSH6	mutS	MSH2	2,771	7q22

on the HNPCC mutation database (www.nfdht.nl). There are no common mutations as such in HNPCC, but a number of founder mutations have been recognised particularly in Finland [55, 56] and in Newfoundland [57].

As with FAP, the identification of causative mutations allows for the provision of pre-symptomatic testing of those individuals at risk. It can be more complicated than that for APC as both nonsense and missense mutations have been identified in both genes. The latter are problematic as it can be difficult to confirm that a change in an amino acid sequence is truly the cause of the disease. The presence of the change in all affected members of the pedigree, a significant alteration in the properties of the wild-type and mutant amino acid, e.g. a hydrophobic substituted for a hydrophilic amino acid, and the degree of conservation of the amino acid in other species can all help to indicate that the change is significant. However, to be certain of the role of the mutation, functional assays are required [58] and these tend to be used primarily in research laboratories. Missense mutations may therefore remain unusable for pre-symptomatic diagnosis.

Several studies have tried to develop a rational, efficient and reasonably cost-effective strategy to screen for MMR mutations given the lack of a clear clinical diagnosis, the

involvement of several genes and the lack of genotype-phenotype correlations as described below. The ultimate goal is to have maximum sensitivity, i.e. as few false negatives as possible along with the maximum specificity. Aaltonen et al. [59] used microsatellite instability as a preliminary screening test of a panel of 509 patients with colorectal cancer. Of the 63 who were positive, 10 had a germ line MMR mutation, thus emphasising the value of MSI as a pre-screen. Wijnen et al. [60] have shown that a younger age at diagnosis, fulfilment of the Amsterdam criteria and the presence of endometrial cancer in the kindred are independent prognosticators of germ line mutations in hMSH2 or hMLH1. They used these to develop a logistic model for estimating the likelihood of a mutation in either gene. Loukola et al. [61] have suggested that a consolidation of these two strategies, i.e. combination of family information plus the presence of MSI, should be considered. The Bethesda criteria described in table 2 were suggested as a method to identify those individuals who should initially be tested for MSI. Those which were positive could then go on to mutation screening [43]. A second study has also shown that adherence to the strict Amsterdam criteria for mutation testing will also miss a number of patients with germ line mutations [62]. Again, a combination of MSI analysis and modified family history criteria was advocated to predict those most likely to have a germ line mutation.

Unlike FAP, there are no very clear genotype-phenotype correlations in HNPCC. Some loose associations have however been made. There is an increased risk of extracolonic cancers in individuals with hMSH2 mutations compared to hMLH1 mutations [63]. hMSH6 mutations have been associated with an atypical phenotype occurring in smaller pedigrees and associated with a later age of onset, a predominance of extracolonic cancers

and a lower incidence of microsatellite instability [52].

An important feature of HNPCC when counselling patients following mutation detection is the incomplete penetrance of the phenotype. Inheritance of a hMSH2 or hMLH1 mutation is associated with an 80% risk of developing colorectal cancer by the age of 80 [63]. Female members of such families have a 40–50% risk of endometrial cancer. The risk of developing ovarian cancer. The risk of developing ovarian cancer is approximately 9%. In at least one study, considerable differences were shown for the risk of developing colorectal cancer depending on the sex of the individual [15]. Females had a much lower risk of colorectal cancer, 30% compared to 74% for males, and in fact the risk of endometrial cancer in females was higher than that for colorectal cancer.

Procedures and Consequences of Genetic Testing for Colorectal Cancers

Screening of patients at risk of colorectal cancer, both clinically and by molecular testing, can clearly prevent the early death of the individual. This is best done by the establishment of a genetic register and the benefits of such a process have been demonstrated for FAP [6, 16]. As mentioned at the beginning of this review, molecular testing of cancer families is ideally performed within the context of genetic centres to provide pre-test counselling and correct interpretation of tests [2]. Studies have shown that this approach has not always been used particularly with respect to pre-test counselling [64].

The effectiveness of various treatment strategies including the use of non-steroidal anti-inflammatory drugs such as sulindac or even aspirin is now under investigation in individuals proven to be APC mutation carriers with a view to try to prevent or slow down the development of polyps [65]. A study

Table 4. Clinical syndromes associated with breast cancer

Site-specific breast cancer
Breast/ovarian cancer
Cowden's disease
Lynch type 2 syndrome
Li-Fraumeni syndrome
Peutz-Jeghers syndrome
Klinefelter syndrome

has shown that the MSI in colorectal cells with an MMR defect is considerably reduced following exposure to sulindac or aspirin [66].

Finally, the cost benefits of genetic testing have also been demonstrated for FAP [67]. Costs of mutation analysis are up to £750 in the UK and up to \$1,300 in the US. Costs of conventional screening by flexible sigmoidoscopy in the absence of genetic testing are around \$3,200. Clearly genotyping can reduce the costs of screening and the larger the pedigree and the more at-risk individuals, the greater the cost advantage.

Familial Breast and Breast/Ovarian Cancer

Approximately 1 in 8 to 1 in 12 women will develop breast cancer and around 5% of all cases are due to an inherited predisposition. The autosomal dominant nature of breast cancer in some families has been recognised for over 100 years. In women under 35 years of age, inherited breast cancer accounts for around 35% of cases, although it accounts for <1% of cases diagnosed over 80 years of age. The syndromes associated with inherited breast cancer are shown in table 4. This section will deal primarily with the two major genes contributing to familial breast and breast/ovarian cancer, namely BRCA1 and BRCA2.

Guidelines for referral and screening in breast cancer families with varying numbers of affected relatives have recently been published [8]. As with other familial cancers, a detailed family history should be obtained and appropriate counselling given before any molecular testing is offered. It is perhaps even more important that this is done for breast cancer than for colorectal cancer given the complex nature of the mutations as described below and the lack of such clear-cut screening methods compared with sigmoidoscopy and colonoscopy.

A gene for familial breast cancer was initially mapped to chromosome 17 in 1990 and was termed BRCA1 [68]. An early study indicated the presence of heterogeneity by demonstrating that the majority of families with breast and ovarian cancer were likely to be caused by this gene, whereas only a proportion of families with breast cancer alone were likely to be caused by BRCA1 [69]. The BRCA1 gene was isolated in 1994 [70] and shown to comprise 24 exons with little homology to other known proteins. Germ line mutations have been identified throughout the gene and over 300 are listed on the breast cancer information core (BIC) database (www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/). In 1994, BRCA2 was mapped to chromosome 13 [71] and was isolated a year later [72]. The gene contains 27 exons. Again a large number of mutations have been identified throughout the gene and are also listed on the BIC database. Existing evidence suggests that at least one or more genes with similar effects to BRCA1 and 2 remain to be detected.

Several approaches have been used to detect mutations in BRCA1 and 2. As the genes are large, it is theoretically necessary to scan 5,283 codons. DNA sequencing is possible and is used in the commercial setting. Most diagnostic laboratories carry out a cascade

system of mutation screening. Regions of the genes in which mutations are clustered are screened first. A multiplex heteroduplex analysis has been developed covering 4 regions of BRCA1 in which 24% of mutations can be found [73]. Following this, exon 11 of BRCA1 and exons 10 and 11 of BRCA2 can be analysed using PTT [74]. Together these tests would be expected to detect around 50% of all mutations in BRCA1 and 2. Further screening of both genes can then be carried out usually by SSCP or DGGE. Once a mutation is identified, presymptomatic testing is possible for at-risk family members (fig. 7). As with other cancers, mutation analysis is possible using DNA extracted from formalin-fixed paraffin-embedded material, but care must be taken when using this approach as anomalous results have been encountered. Although some BRCA1 and 2 mutations listed in the database have been seen on more than one occasion (including founder mutations described below), most are private. In both genes, nonsense and missense mutations have been identified, the latter being problematic as it can be difficult to prove that they are truly causative as was described above for HNPCC.

In genetically isolated populations, a number of founder mutations have been found. In the Ashkenazi Jewish population, one mutation, 185delAG, has been found in 20% of women with early onset breast cancer and has a carrier frequency of 0.9% in the Ashkenazi population [75]. This is in contrast to the general population where the prevalence of all BRCA1 mutations is around 0.2%. Further studies have identified two other common mutations in the Ashkenazi population. 5382insC in BRCA1 was found at a carrier frequency of 0.13% and 6174delT in BRCA2 was found at a carrier frequency of 1.52% [76]. In total, 60% of Ashkenazi Jewish women with early onset ovarian cancer and 30%

Fig. 7. Pedigree of a family with breast cancer. Individual II-3 developed breast cancer at age 50, III-2 had breast cancer at 37 years, III-7 at 35 years and IV-2 at 27 years. At-risk offspring of the affected individuals can be offered presymptomatic testing once the causative mutation is identified. No evidence of breast cancer has been detected in individual II-2, indicating the non-penetrant nature of BRCA mutations.

with early onset breast cancer carry one of these three mutations [77]. Given the frequency of these mutations in the Ashkenazi population, it is not surprising that double heterozygotes have also been found. 995del5 in BRCA2 has been shown to be a founder mutation in the Icelandic population where, in addition to female breast cancer, it also accounts for 40% of male breast cancer detected over the last 40 years [78]. Two BRCA1 genomic deletions comprising 36% of all families with a BRCA1 mutation have been shown to be founder mutations in the Dutch population [79]. Two mutations, C4446T in BRCA1 and 8765delAG have been identified as founder mutations in the French Canadian population [80]. From these studies it can be seen that it may be important to know the ethnic origins whenever possible if carrying out mutation detection in familial breast cancer.

Some genotype-phenotype correlations have been described in BRCA1 and 2, which can help with the detection of mutations. However, as they are by no means absolute, they should not be used for genetic counselling purposes. In general, the presence of ovarian cancer is more likely to indicate the presence of a BRCA1 mutation. Within BRCA1, mutations in the 3' end of the gene are associated with a lower risk of ovarian cancer [81]. If ovarian cancer is seen in BRCA2 families, the causative mutations are clustered in a 3.3-kb region of exon 11 bordered by nucleotides 3035 and 6629 [82]. Male breast cancer is generally associated with BRCA2 mutations [83]. Pancreatic, laryngeal and prostate cancer have also been associated with BRCA2 mutations [84].

Counselling for those at risk of familial breast cancer is particularly difficult because

of the reduced penetrance of BRCA1 and 2 mutations and the reduced efficacy of clinical screening. Risks of developing breast cancer for carriers of a BRCA1 mutation have been calculated to be 51% by age 50, rising to 85% by 70 years of age. The risk of ovarian cancer for BRCA1 carriers is approximately 30% by age 60 [85]. The risk of breast cancer for carriers of a BRCA2 mutation is 60% by age 50 and 71% by age 60 [83, 84]. The risk of male breast cancer for BRCA2 carriers is 6.3% by age 70. Female carriers of BRCA mutations have been identified who remain healthy with no evidence of cancer over 80 years of age. This information needs to be made clearly available to patients undergoing predictive testing and who may be considering prophylactic mastectomy.

There are many benefits to families from testing for BRCA mutations, but there are also drawbacks. Once a high-risk family has been referred to a genetic clinic it should be made aware to them that it might not be possible to identify a mutation. There are a number of causes for this including (a) lack of a DNA sample from an affected individual – at the present time mutation screening of unaffected at-risk individuals is not recommended [6], (b) only two genes have been isolated which are screened for mutations, so a high-risk family may have a mutation in an as yet unidentified gene, (c) mutation detection is not 100% sensitive. Once mutations are found, predictive tests are offered and results given back to patients, there remain difficult choices for the patient including screening options, preventative surgery such as prophylactic mastectomy and oophorectomy or chemoprevention. Follow-up of patients and the availability of psychological support are therefore important. Several psychological studies of the benefits of molecular testing in breast cancer are currently under way in the UK. It must also be remembered that there

are significant cost benefits if mutations can be identified in high-risk families. The cost of a molecular test to identify a mutation is around £500–700 in the diagnostic setting and \$2,500 in the commercial setting. The costs of predictive testing are then around £100 per person. This is minimal compared to the cost of regular MRI surveillance of women at risk of inherited breast cancer.

Multiple Endocrine Neoplasia

Multiple Endocrine Neoplasia Type 1 (MEN-1)

MEN-1 is an autosomal dominant condition associated with malignancies of the endocrine tissues including the parathyroids, the endocrine pancreas and the anterior pituitary. There is considerable inter- and intrafamilial variation in the phenotype. The age-related penetrance of the disease is 100% by age 60 [86]. Biochemical screening can detect disease approximately 10 years before patients are symptomatic. Screening of at-risk individuals is important as many of the complications of the condition can be treated. This should begin around 10 years of age and be continued at 5-yearly intervals and include measurement of serum calcium levels, parathyroid hormone levels, pituitary hormone levels, and pancreatic hormones as well as routine clinical investigations. Treatment for tumours is normally surgical.

The gene was initially mapped to chromosome 11q13 [87]. It took a further 9 years before the gene was eventually isolated [88] and shown to have 10 exons. It encodes a 610-amino acid protein called menin. Mutations have been found in exons 2–10 (exon 1 being non-coding) and are primarily nonsense mutations or insertions and deletions predicted to produce a truncated protein resulting in the loss of function of menin [86, 89]. The exact

role of missense changes remains to be clarified [90]. Germ line mutations have been found in most cases of familial MEN1 as well as the majority of sporadic cases. At the present time there are no genotype-phenotype correlations. Once individuals have been identified as gene carriers, clinical and biochemical evaluation can be commenced after the age of 5 years as described [91]. This will enable early detection of tumours and thereby allow appropriate management of the patient.

Multiple Endocrine Neoplasia Type 2 (MEN-2)

MEN2 is characterized by the presence of medullary thyroid cancer (MTC) in association with pheochromocytoma. There are three clinical variants, MEN2A, MEN2B and familial medullary thyroid cancer (FMTC). MEN2A is the most common variant and is associated with pheochromocytoma and parathyroid tumours as well as MTC. In MEN2B, parathyroid involvement is rare. FMTC is characterised by the presence of MTC alone [92]. All forms may be inherited as an autosomal dominant disorder, but may also occur sporadically. Tumours associated with the disorders can be detected by biochemical screening and this can result in diagnosis and hence reduce morbidity and mortality. Screening depends on the measurement of plasma calcitonin in both the basal state and following pentagastrin or calcium stimulation and should be carried out from 1 year of age until at least 30 years [92]. The test, however, is unpleasant and false positives do arise. Clarification of the status of at-risk individuals can be difficult so the use of a DNA test to identify gene carriers is still valuable.

The gene for MEN2 was mapped to the pericentromeric region of chromosome 10 in 1987 [93] and initially it was assumed that the gene responsible would be a tumour suppres-

sor gene as is the case for the majority of other familial cancer syndromes. However, the candidate gene based on genetic and physical mapping studies was the RET gene, which is a transforming oncogene. Several laboratories investigated RET and identified mutations in cases of MEN2A, 2B and FMTC [94, 95]. In MEN2A, mutations in 5 cysteine residues of the extracellular domain of RET were found in 93% of cases, with mutations at codon 634 being present in 83% [94, 96]. A strong genotype-phenotype correlation was found between the mutation at codon 634, particularly a cysteine-to-arginine change, and the presence of pheochromocytoma [96]. A single missense mutation at codon 918 in which methionine is replaced by threonine is found in over 90% of cases of MEN2B. FMTC is associated with the same spread of mutations as in MEN2A, but the incidence of mutations is more evenly spread across the five cysteines. In all three conditions, the effect of the mutations is to confer a gain of function to the protein. However, the mutations achieve their effects by different mechanisms as would be expected from the differences in the phenotype between the three disorders [97]. Interestingly, a loss of function mutation is also seen in RET in which the gene is inactivated or there is abrogation of the function of the RET protein. These mutations cause a completely unrelated disorder, Hirschsprung's disease, in which there is an abnormality of the hindgut characterised by the absence of enteric autonomic ganglia [98].

Other Familial Cancer Syndromes

Von Hippel-Lindau Disease (VHL)

This is an autosomal dominant disorder with an incidence of around 1 in 35,000 [99]. It is associated with a wide variety of tumours including retinal angiomas, cerebellar, spinal

cord and brain stem haemangioblastomas, renal cell carcinoma and phaeochromocytoma [99]. The penetrance of the disease is around 90% by 60 years of age. Systematic screening and early detection of tumours have been shown to reduce morbidity and mortality [100]. Screening of both affected and at-risk individuals is fairly intensive involving annual examinations, direct and indirect ophthalmoscopy, MRI scans at 3-yearly intervals, annual renal ultrasound scans and annual 24-hour urine collectin for VMA's [100].

The gene for VHL was localised to 3p25–26 [101, 102] and isolated in 1993 [103]. This enabled presymptomatic diagnosis of at-risk individuals and removal of those at negligible risk from the intensive screening otherwise necessary. The gene is relatively small compared with many of the others described above, with only 3 exons encoding a 213-amino acid protein. The identification of at least five VHL binding proteins has led to a clearer understanding of the function of VHL itself [104]. The VHL protein (a) regulates the rate of transcription elongation by binding to elongin B and C, (b) down-regulates expression of vascular endothelial growth factor mRNA, (c) binds to CUL2, a member of the cullin binding proteins which are believed to target certain proteins for ubiquitination and degradation, (d) is involved in cell cycle control and finally (e) is involved in fibronectin metabolism [104].

Over 150 mutations have been identified in VHL in all three exons. Approximately 60% of mutations are nonsense and missense mutations whereas the remainder are whole gene or partial gene deletions [105–108]. A combination of direct sequencing with or without SSCP for detection of nonsense and missense mutations plus either Southern blotting or quantitative fluorescent PCR is capable of detecting close to 100% of mutations [108, plus personal communications]. Many

mutations identified are different in different families. Founder mutations in VHL are uncommon but there are a small number of recurring mutations [105–107].

Some genotype-phenotype correlations do exist. A major phenotypic difference between VHL families is the presence or absence of phaeochromocytomas. Nearly all families without phaeochromocytomas have deletions or nonsense mutations. The presence of phaeochromocytomas is associated in >90% of cases with missense mutations [106, 107]. In particular a missense mutation occurring at codon 238 (renumbered as codon 167 in later papers) has been identified in 43% of these families. This finding suggests that a mutant protein must be full length to produce phaeochromocytomas. Mutation-specific counselling, however, still has to be guarded as the total number of cases identified remains small.

Li-Fraumeni Syndrome

This is a rare autosomal dominant condition characterised by the presence of a range of tumours including breast cancer, brain tumours, soft tissue sarcomas, osteosarcomas, leukaemias and adrenocortical tumours [109]. The condition is defined by very strict clinical criteria [110], but a group of families with a Li-Fraumeni-like condition have also been described [111]. More than 90% of gene carriers are expected to develop tumours by age 70 [112]. It is caused by mutations in the tumour suppressor gene TP53. Mutations are located primarily in exons 5–8, although they have also been found in other exons [113]. Mutations in TP53 have been found in 70% of all families with Li-Fraumeni syndrome and 20% of Li-Fraumeni-like families when the entire gene including non-coding regions is sequenced [113]. Although identification of mutations in TP53 allows presymptomatic diagnosis to be offered to at-risk relatives, the

lack of effective screening for the majority of associated tumours make testing for this condition very complex and ethically difficult.

Neurofibromatosis

The neurofibromatoses are a group of neurocutaneous syndromes affecting tissues derived from the neural crest. Two clinically distinct forms have been recognised: neurofibromatosis type 1 (NF1) and neurofibromatosis type 2 (NF2).

NF1 is a common autosomal disorder affecting 1 in 3,000 to 1 in 5,000 people. It is characterised by multiple café au lait spots, neurofibromas and Lisch nodules of the iris [114]. Malignancies including optic gliomas, neurofibrosarcomas, brain gliomas, pheochromocytomas and leukaemias are seen, but are less common complications of the disorder. There is a very wide variation in expression of the disease. The gene was mapped to chromosome 17 in 1987 and cloned 3 years later [115]. Mutation analysis has proved very difficult, although over 100 mutations have now been identified, the majority of which are expected to cause truncations of the protein product, neurofibromin [116]. The demand for molecular testing is relatively low for two reasons. The majority of cases of NF1 can be established on clinical criteria even in early childhood, so presymptomatic diagnosis on molecular grounds is not a very high priority. As the phenotype is highly variable and mutation analysis cannot be used to predict the severity of the disease, prenatal diagnosis is rarely taken up [116].

NF2 is an autosomal dominant disorder with an incidence of approximately 1 in 40,000 and is characterised by the presence of bilateral acoustic neuromas. There is also a predisposition to other CNS tumours including meningiomas and astrocytomas [117]. Early diagnosis and treatment can prevent the neurological impairment but hearing is rarely

preserved. At risk relatives can be monitored by regular audiometry and skin and eye examinations with MRI scans at intervals. The gene has been mapped to chromosome 22 and isolated in 1993 [118]. The protein product of the gene has been called merlin. Most of the mutations cause the synthesis of a truncated protein. An association between nonsense and frameshift mutations and clinical manifestations has been made. Mutations that cause premature truncation of the NF2 protein present with severe phenotypes [119]. In contrast, mutations that affect only a single amino acid have mild NF2. Subjects with truncating mutations have been shown to be significantly more likely to develop symptoms before 20 years of age and to develop at least 2 symptomatic CNS tumours in addition to vestibular schwannomas before 30 years of age [120]. Presymptomatic and prenatal diagnosis is therefore useful in the management of this condition.

Gorlin Syndrome

Gorlin syndrome or basal cell naevus syndrome is a relatively rare (approximately 1:57,000), autosomal dominant disorder associated with multiple basal cell carcinomas of the skin, and palmar and plantar pits [121]. In addition there are many other associated features including frontal and parietal bossing, prominent jaw, odontogenic keratocysts of the jaw, calcification of the falx and bifid, absent or rudimentary ribs. Surveillance is by annual dermatological investigations and regular monitoring of jaw cysts which can erode locally if left untreated. Radiation increases the incidence of the basal cell naevi so should not be used. Basal cell naevi and jaw cysts are present in 90% of gene carriers by age 40 [122]. The gene was mapped to chromosome 9q in 1992 [123] and was isolated in 1996 [124]. The gene is *PTCH* and mutation analysis has identified many mutations throughout

the coding region, the majority of which are truncating [125]. No genotype-phenotype correlations have so far been found.

Familial Gastric Cancer

Gastric cancer is a major cause of death from cancer world-wide. Around 10% of cases show familial clustering. In 1998, linkage in a large Maori pedigree was found to chromosome 16q21 [126]. The E cadherin gene (CDH1) lay within this region and SSCP analysis of the gene identified a band shift in exon 7 which when sequenced proved to be a splice site mutation. The consequence of this mutation was to alter splicing and introduce a premature stop codon. Analysis of two further families with early onset, histologically diffuse gastric cancer identified additional mutations in CDH1. E cadherin is a cell adhesion molecule which binds to β -catenin at adherence junctions preventing cell signalling. Loss of E cadherin results in increased cell mobility. A second study of non-Maori families selected on the basis of either 2 cases of gastric cancer in first-degree relatives with one affected before age 50 years, or 3 or more cases of gastric cancer, identified mutations in 25% of cases confirming a role for E cadherin in familial gastric cancer [127]. In addition, 1 of the mutation carriers in this study developed colorectal cancer at age 30, indicating that CDH1 may also be implicated in some cases of early onset colorectal cancer. However, only a proportion of familial gastric cancers can be accounted for by CDH1 mutations and other genes remain to be identified. Guidelines for the management of familial gastric cancers have recently been developed [128].

Hereditary Prostate Cancer

Prostate cancer is a major health problem accounting for over 8,000 deaths per year in the UK, and in the US is the second most common cause of death from cancer. A strong

hereditary component to prostate cancer has been postulated. A gene for familial prostate cancer has been localised to chromosome 1q24–25 and named HPC1 [129]. However there is clear evidence of locus heterogeneity and other possible candidate genes have been identified [129].

Conclusion

This review has highlighted the benefits of mutation screening and presymptomatic diagnosis in families with a strong history of cancer. Further genes are likely to be identified in the immediate future, especially with the recent publication of the entire sequence of the human genome. This will mean increased benefits for patients with the ability to target screening to high-risk individuals. In addition, there will be cost benefits to health services. As well as benefiting those individuals with familial cancers, it will potentially offer insight into the molecular defects in sporadic cancer, and alternative chemotherapeutic options should also become available. The first 10 years of this millennium are therefore likely to bring many advances in the field of cancer research and molecular diagnostics.

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